Inflammation-Related Gene Polymorphisms and Colorectal Adenoma

Marc J. Gunter,[1,2] Federico Canzian,[3,4] Stefano Landi,[3,5] Stephen J. Chanock,[1] Rashmi Sinha,[1] and Nathaniel Rothman[1]

[1]Division of Cancer Epidemiology and Genetics, Department of Health and Human Services, National Cancer Institute, NIH, Bethesda, Maryland; [2]Department of Epidemiology and Population Health, Albert Einstein College of Medicine, Bronx, New York; [3]IARC, Lyon, France; [4]German Cancer Research Center, Heidelberg, Germany; and [5]Department of Biology and Genetics, University of Pisa, Pisa, Italy

Abstract

Chronic inflammation has been reported to be a risk factor for colorectal neoplasia. The propensity to mount an inflammatory response is modified by germ line variation in cytokine and other inflammation-related genes. We hypothesized that a proinflammatory genotype would be positively associated with colorectal adenoma, a precursor of colorectal cancer. We investigated the association of colorectal adenoma with 19 single nucleotide polymorphisms in a range of important proinflammatory (*IL1B, IL6, IL8, TNF*, and *LTA*) and anti-inflammatory (*IL4, IL10*, and *IL13*) cytokines and other inflammation-related genes (*PTGS2* and *PPARG*) in a case-control study of risk factors for colorectal polyps in which all participants (ages 18-74 years) had undergone colonoscopy or sigmoidoscopy. The study sample comprised 244 cases of colorectal adenoma and 231 polyp-free controls. Compared with being homozygous for the common allele, heterozygosity at the *IL1B* −31 (C>T) locus was associated with an odds ratio (OR) for colorectal adenoma of 1.8 [95% confidence interval (95% CI), 1.2-2.9]. Homozygous carriers of the *IL8* −251-A allele were at 2.7-fold increased risk of adenoma (95% CI, 1.5-4.9) compared with homozygosity for the common T allele, whereas carriage of at least one *IL8* −251-A allele conferred a 1.5 increased odds of disease (95% CI, 1.0-2.4). Among non−nonsteroidal anti-inflammatory drug users, there was a statistically significant association between the *IL10* −819-T/T genotype and adenoma compared with the common *IL10* −819-C/C genotype (OR, 3.9; 95% CI, 1.1-13.6), which was not evident among nonsteroidal anti-inflammatory drug users (OR, 0.7; 95% CI, 0.3-1.5; $P_{\text{interaction}}$ = 0.01). These exploratory data provide evidence that polymorphic variation in genes that regulate inflammation could alter risk for colorectal adenoma. (Cancer Epidemiol Biomarkers Prev 2006;15(6):1126−31)

Introduction

Both mechanistic and observational data implicate chronic inflammation in the etiology of colorectal cancer. At the cellular level, the colonic epithelium is exposed to a range of toxic and pathogenic challenges, including the balance between intestinal microflora. In turn, a shift in microflora can result in a change in immune response, including the induction of inflammation (1). A notable hallmark is the release of proinflammatory cytokines by infiltrating lymphocytes. These can lead to the generation of reactive oxygen species and other genotoxic compounds in the epithelial environment. Cytokines are peptide mediators that act between immunocompetent and hematopoietic cells and between the immune and neuroendocrine systems. They are synthesized by activated cells and exert their biological activities upon binding to specific receptors expressed on target cells with subsequent proinflammatory or anti-inflammatory consequences (see Table 1). There is evidence to suggest that cytokines are involved in the control of cancer development, and that they may be relevant for gastrointestinal tumors. The genes that encode these peptides are polymorphic, and the most common variant is the single nucleotide polymorphism (SNP), of which many have been identified in the regulatory regions of cytokine genes. For example, the *IL1B −1060-T* allele, which lies in the promoter region of this proinflammatory cytokine, is associated with enhanced interleukin-1β (IL1β) expression and has been linked to increased risk of gastric cancer in response to *Helicobacter pylori* infection (2, 3). Furthermore, common variants of *IL6, IL8*, and *IL10* have been associated with colorectal cancer risk (4, 5).

Stimulation of arachidonic acid metabolism is a critical step in the induction of inflammation. Prostaglandin H synthase (PTGS; also known as cyclooxygenase) catalyzes the conversion of arachidonic acid to prostaglandin precursors, which function as autocrine and paracrine mediators of a range of cell functions, including vasodilation and nociception. This enzyme is the pharmacologic target for nonsteroidal anti-inflammatory drugs (NSAID), and inhibition of PTGS2 accounts for at least some of the anti-inflammatory properties of these drugs. Elevated levels of PTGS2, the inducible form of the enzyme, have been shown in colon cancer tissue (6), and this enzyme has proangiogenic, pro-proliferative, and antiapoptotic effects (7). Allelic variants of *PTGS2* have been associated with colorectal neoplasia in some (8-10) but not all studies (11, 12). It was recently reported that a variant within the *PTGS2* promoter region has been associated with colorectal adenoma among non-NSAID users (8).

The peroxisome proliferator-activated receptor γ (PPARγ) is a nuclear receptor that functions as a transcriptional regulator of metabolism. Implicated in the pathophysiology of obesity and insulin resistance, PPARγ binds small molecules, such as fatty acids, and is required for the accumulation of adipose tissue. PPARγ also possesses anti-inflammatory properties. It has been suggested that PPARγ binds nuclear factor-κB, activator protein 1, and signal transducers and activators of transcription factors, thereby inhibiting initiation of the inflammatory response (13). Natural ligands and drug agonists

Received 1/23/06; revised 3/3/06; accepted 4/4/06.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Marc Gunter, Department of Epidemiology and Population Health, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461. E-mail: mgunter@aecom.yu.edu

doi:10.1158/1055-9965.EPI-06-0042

Table 1. List of studied genes and SNPs

Gene	Function	Polymorphism	dbSNP no.	Variant phenotype
IL1B	Proinflammatory, primary initiator of inflammatory response	−31C>T	rs1143627	C allele: increased expression (2)
		Ex5+14C>T	rs1143634	
IL4	Regulates antibody production, attenuates inflammatory response	−584C>T	rs2243250	T allele: Increased transcriptional activity (29, 30)
		−1098G>T	rs2243248	
IL5	Hematopoietic growth factor, promotes growth and differentiation of eosinophils	−745C>T	rs2069812	
IL6	Proinflammatory, initiator of inflammatory response, growth factor for cancer cells	−174C>G	rs1800795	G allele: increased expression (31)
IL8	Proinflammatory chemokine, activates neutrophils	−251A>T	rs4073	A allele: increased expression (22)
IL10	Anti-inflammatory, regulates T-cell and macrophage function	−819C>T	rs1800871	In LD with −592 C>A SNP which is linked to reduced expression (27, 28)
		−1082A>G	rs1800896	A allele: reduced expression
IL13	Anti-inflammatory, suppresses IL1, IL6, and IL8 production	−1069C>T	rs1800925	T allele: increased expression (32)
		IVS3−24T>C	rs1295686	
LTA	Proinflammatory, stimulates prostaglandin synthesis, induces reactive oxygen species production in neutrophils	IVS1−82G>C	rs746868	
		IVS1+90G>A	rs909253	
TNF	Proinflammatory, key immunomediator	−308A>G	rs1800629	A allele: higher transcriptional activation (33, 34)
PPARG	Anti-inflammatory, tumor suppressor and promoter properties	Ex4−49C>G (Pro^{12}Ala)	rs1801282	Ala allele: reduced ligand binding (35)
PTGS2	Converts arachidonic acid to prostaglandins, thereby promoting inflammatory response	−765C>G	rs20417	C allele: reduced expression (36)
(*COX2*)		Ex3−8G>C	rs5277	
		IVS5−275G>T	rs20432	
		Ex10+837C>T	rs5275	

of PPARγ have been shown to reduce intestinal inflammation in both humans and rodent models (14, 15). Furthermore, the formation of aberrant crypt foci by chemical induction is inhibited by PPARγ ligands (16). A common *PPARG* SNP in exon 12 that leads to a nonsynonymous amino acid substitution has been associated with colorectal cancer and adenoma (4, 17).

Based on emerging evidence that chronic inflammation is related to colorectal neoplasia, and that the expression and function of a number of important cytokines and other inflammation-related enzymes is under genetic control, we hypothesized that a proinflammatory genetic profile is associated with increased susceptibility to colorectal adenoma, an established precursor of colorectal cancer. We investigated the relation between 19 polymorphisms in *IL1B, IL4, IL5, IL6, IL8, IL10, IL13, LTA*, and *TNF* as well as the *PTGS2* and *PPARG* genes with colorectal adenoma.

Materials and Methods

Study Sample. We genotyped participants from a case-control study of colorectal adenomas conducted at the National Naval Medical Center (Bethesda, MD); cases were patients who were diagnosed with colorectal adenoma at sigmoidoscopy (18%) or colonoscopy (82%) between April 1994 and September 1996 (details of this study are described elsewhere; refs. 18, 19). Controls were selected among individuals confirmed as polyp-free during sigmoidoscopic screening and were frequency matched to cases by age (±5 years) and gender. To be eligible for the study, cases and controls had to be residents of the study area, between ages 18 and 74 years, and had never been diagnosed with Crohn's disease, ulcerative colitis, colorectal neoplasms, or cancer except nonmelanoma skin cancer.

The participation rates were 84% for the cases (244 of the 289 eligible cases identified) and 74% for the controls (231 of 314 eligible controls). The main reason for nonparticipation was subject refusal (12% of cases and 21% of controls) followed by illness (3% of cases and 4% of controls). Three cases with familial adenomatous polyposis syndrome were excluded from the study. All 231 control subjects had been verified by sigmoidoscopy. Of the 244 cases, 210 (86.1%) had undergone colonoscopic examination, and 34 (13.9%) had undergone sigmoidoscopic examination. The average time between colorectal examination and blood draw was 3 days for cases and 23 days for controls, and the time between colorectal examination and collection of questionnaire data was 2.4 and 3.3 months for cases and controls, respectively.

Genotyping and Statistical Analysis. SNPs were selected on the basis of functional data related to changes induced in the expression of the cytokine and prevalence (>5% minor allele frequency in Caucasians). For *PTGS2*, a SNP in a putative promoter sequence variant that has been associated with differential PTGS2 expression was genotyped. In addition, three SNPs with >5% prevalence in Caucasian populations and spaced at approximately regular intervals across the gene region were selected for genotyping. A SNP that gives rise to a nonsynonymous amino acid change in PPARγ and has been associated with colorectal neoplasia was also genotyped (see Table 1). Genotyping of *IL6, IL8, PPARG*, and *PTGS2* was performed according to the methodology of Landi et al. (4). Primer sequences for the *PTGS2* genotyping probes are available upon request. All remaining genotyping was done using Taqman assays, and detailed protocols can be accessed at http://snp500cancer.nci.nih.gov/assays. To ensure reproducibility of genotyping methods, multiple blinded quality control samples (n = 30) from two individuals were embedded among the case-control samples. For all genotypes tested, the quality control samples indicated a reproducibility rate of 100%.

Differences in genotype distributions between cases and controls were ascertained by the χ^2 statistic. Association between genotypes and colorectal adenoma were calculated as odds ratios (OR) with 95% confidence intervals (95% CI) by unconditional logistic regression and were adjusted for age, sex, and ethnicity using SAS software (version 8.2). In all cases, homozygosity for the most common allele in Caucasians was used as the reference category. In addition, assuming a dominant model of inheritance, heterozygotes and homozygosity for the less common allele categories were collapsed to create a less common allele carrier category. For genes or chromosomal regions genotyped for more than one SNP, linkage disequilibrium (as determined by the D' statistic) between adjacent markers was estimated and when applicable, haplotypes reconstructed from unphased genotype data using PHASE version 2.0 (20). Overall differences in haplotype distribution between cases and controls were assessed using the likelihood ratio test statistic. All P-values were two sided, and all models were adjusted for age, sex, and ethnicity. To account for the large number of comparisons made in this study, the false-positive report probability was calculated using a prior probability of association using the method of Wacholder et al. (21).

Results

In total, 244 cases of colorectal adenoma and 231 polyp-free controls participated in this study. Demographic and other selected characteristics of the study population are presented in Table 2. The distribution of gender differed among the case and control groups; males were overrepresented in the case category. The median age of cases and controls were 60 and 57 years, respectively. Overall, cases had a higher body mass index (P = 0.04) and were more likely to be current smokers (P = 0.01) compared with controls. Regular use of NSAIDs was more common among controls compared with cases (P = 0.002). Inclusion of body mass index, NSAID use, and smoking status did not cause a material change in the risk estimates; thus, these variables were not included as covariates in the final regression models.

Among the control group, all genotypes were distributed in accordance with Hardy-Weinberg equilibrium except for two SNPs: *IL1B* −31 (P = 0.004) and *IL10* −1082 (P = 0.003). Table 3 provides genotype distributions and ORs for possible associations between genotypes and colorectal adenoma. Compared with homozygosity for the common allele, heterozygosity at the *IL1B* −31 (C>T) locus was associated with an OR for colorectal adenoma of 1.8 (95% CI, 1.2-2.9), and carriage of an *IL1B* −31-C allele was associated with a 1.5-fold increased risk of colorectal adenoma (95% CI, 1.0-2.3). Homozygous carriers of the *IL8* −251-A allele were at 2.7-fold increased risk of adenoma (95% CI, 1.5-4.9) compared with homozygosity for the common T allele, whereas carriage of at least one *IL8* −251-A allele conferred a 1.5 increased odds of disease (95% CI, 1.0-2.4). There was a suggestion of a positive association between the *IL6* −174-C/C genotype and adenoma when compared with the common G/G genotype, but this did not attain statistical significance (OR, 1.5; 95% CI, 0.8-2.9). The *IL10* −819-T/T genotype conferred a 2.1-fold increased risk of adenoma when compared with the common *IL10* −819-C/C genotype, but this was not statistically significant (95% CI, 0.9-4.8). There was an indication that the *TNF* −308 A/A and *LTA* IVS1 +90 G/G genotypes were associated with colorectal adenoma, although the ORs did not quite attain statistical significance (Table 3). There was no suggestion of association between the remaining genotypes and colorectal adenoma risk.

Table 2. Characteristics of the study population

Characteristic	Cases (n = 244)	Controls (n = 231)	P*
Females (%)	22.5	36.4	0.0009 †
Age (y)	60 (55.5-74.5)	57 (43-71)	0.07
Non-White (%)	11.1	9.1	0.48 †
Body mass index (kg/m^2)	26.5 (21.9-31.2)	25.8 (21.1-30.5)	0.04
Smoking status			
Current smoker (%)	11.1	4.8	0.01 †
Regular smoker (y)	26 (6-46)	18 (0-36)	0.0001
Cigarettes per day	20 (0.8-39.2)	20 (0-40)	0.012
NSAID use ‡ (%)	51.6	65.4	0.002 †
Family history of colon cancer (%)	16.8	11.9	0.13 †

NOTE: All values are medians (interquartile range) unless otherwise indicated.
*Ps derived from the Wilcoxon signed rank sum test unless otherwise indicated.
†P derived from the χ^2 test.
‡Denotes regular use of aspirin or other NSAIDs.

A combinatorial analysis was also employed to investigate the combined effects of the genotypes. Compared with homozygosity for the common allele at both the *IL1B* −31 and *IL8* −251 loci, carriage of an *IL1B* −31-C allele combined with homozygosity for the less common A allele at the *IL8* −251 locus conferred an OR of 5.7 (95% CI, 2.1-15.0; data not shown).

For genes or chromosomal regions with multiple-typed markers (*PTGS2, IL1B, IL4, IL13*, and the *TNF-LTA* gene region), a haplotype-based analysis was employed to test for specific *cis* effects. Estimated haplotypes were not statistically associated with colorectal adenoma in this study (haplotype frequencies available upon request).

As an additional exploratory analysis, the association between genotype and haplotype frequency and colorectal adenoma was investigated among users and nonusers of NSAIDs. Among non-NSAID users, there was a statistically significant association between the *IL10* −819-T/T genotype and adenoma compared with the common *IL10* −819-C/C genotype (OR, 3.9; 95% CI, 1.1-13.6), which was not evident among NSAID users (OR, 0.7; 95% CI, 0.3-1.5), and a statistically significant multiplicative interaction was observed (P = 0.01).

Discussion

We observed statistically significant associations between alleles of proinflammatory cytokines *IL1B* and *IL8* and colorectal adenoma. Furthermore, we identified a significant interaction between a polymorphism in the anti-inflammatory cytokine *IL10* and NSAID use in colorectal adenoma risk.

Although the findings presented here are exploratory, the study is limited by its small size and the relatively large number of statistical comparisons, which together increase the probability that false positives have been featured. For the *IL1B* −31-C and *IL8* −251-A alleles, we had relatively high prior probabilities that they would be associated with colorectal adenoma, based on findings for other cancer sites together with preliminary evidence that the tested variants could have functional consequences (2-4, 22). We applied the method of Wacholder et al., in which the false-positive report probability is calculated using a prior probability of association, observed P, and the statistical power to estimate the validity or ''noteworthiness'' of the findings (21). For carriage of the *IL1B* −31-C and *IL8* −251-A alleles, we calculated that the false-positive report probability associated with the risk estimates for these alleles would be in excess of the moderate prior probability (0.1) that a true association exists. Therefore, we are cautious in our interpretation of these data. However, we view these findings as exploratory and given the emerging evidence on chronic inflammation and colorectal carcinogenesis and the apparent functional role these cytokines play in the innate immune response; further study of these variants in relation to colorectal polyps is warranted.

In addition to statistical bias, additional bias may also lie in study design. In total, 86% of the cases underwent a full

Table 3. Genotype distributions and genotypic risks for association with colorectal adenoma for cytokine and other inflammation-related genes

Genotype	Cases, *n* (%)	Controls, *n* (%)	OR* (95% CI)
IL1B −31			
T/T	74 (37.6)	92 (48.2)	1.0
C/T	99 (50.2)	68 (35.6)	1.8 (1.2-2.9)
C/C	24 (12.2)	31 (16.2)	0.9 (0.5-1.7)
P_{trend} = 0.01			
C-carrier	123 (62.4)	99 (49.8)	1.5 (1.0-2.3)
IL1B Ex5 +14			
C/C	131 (59.0)	129 (61.7)	1.0
C/T	78 (35.1)	66 (31.6)	1.1 (0.7-1.7)
T/T	13 (5.9)	14 (6.7)	0.9 (0.4-2.0)
P_{trend} = 0.82			
T-carrier	91 (41.0)	80 (38.3)	1.1 (0.7-1.6)
IL4 −584			
T/T	132 (68)	114 (64.8)	1.0
C/T	53 (27.3)	53 (30.1)	0.9 (0.5-1.4)
C/C	9 (4.6)	9 (5.1)	0.8 (0.3-2.6)
P_{trend} = 0.86			
C-carrier	60 (31.9)	62 (35.2)	0.8 (0.5-1.4)
IL4 −1098			
T/T	177 (84.3)	156 (82.1)	1.0
G/T	33 (15.7)	32 (16.8)	0.9 (0.5-1.5)
G/G	0 (0)	2 (1.1)	
P_{trend} = 0.91			
G-carrier	33 (15.7)	34 (17.9)	0.9 (0.5-1.5)
IL5 −74			
C/C	94 (44.8)	82 (43.2)	1.0
C/T	92 (43.8)	85 (44.7)	0.9 (0.6-1.5)
T/T	24 (11.4)	23 (12.1)	0.8 (0.4-1.8)
P_{trend} = 0.90			
T-carrier	116 (55.2)	108 (56.8)	0.9 (0.6-1.4)
IL6 −174			
G/G	79 (38.7)	83 (43.7)	1.0
C/G	90 (44.1)	81 (42.6)	1.1 (0.7-1.8)
C/C	35 (17.2)	26 (13.7)	1.5 (0.8-2.9)
P_{trend} = 0.40			
C-carrier	125 (61.3)	107 (56.3)	1.2 (0.8-1.9)
IL8 −251			
T/T	52 (25.4)	65 (34.0)	1.0
A/T	87 (42.4)	94 (49.2)	1.2 (0.7-1.9)
A/A	66 (32.2)	32 (16.8)	2.7 (1.5-4.9)
P_{trend} = 0.002			
A-carrier	153 (74.6)	126 (66.0)	1.5 (1.0-2.4)
IL10 −819			
C/C	125 (56.3)	117 (56.0)	1.0
C/T	76 (34.2)	79 (37.8)	1.0 (0.6-1.5)
T/T	21 (9.5)	13 (6.2)	2.1 (0.9-4.8)
P_{trend} = 0.19			
T-carrier	97 (43.7)	92 (44.0)	1.1 (0.7-1.6)
IL10 −1082			
A/A	61 (27.5)	55 (26.6)	1.0
A/G	114 (51.4)	123 (59.4)	0.8 (0.5-1.3)
G/G	47 (21.1)	29 (14.0)	1.3 (0.7-2.4)
P_{trend} = 0.17			
G-carrier	161 (72.5)	152 (73.4)	0.9 (0.6-1.4)
IL13 −1069			
C/C	120 (61.5)	102 (59.7)	1.0
C/T	67 (34.4)	58 (33.9)	1.0 (0.6-1.6)
T/T	8 (4.1)	11 (6.4)	0.7 (0.2-1.8)
P_{trend} = 0.69			
T-carrier	75 (38.5)	69 (40.3)	1.0 (0.6-1.5)
IL13 IVS3 −24			
C/C	128 (61.5)	110 (57.0)	1.0
C/T	70 (33.7)	73 (37.8)	0.8 (0.5-1.2)
T/T	10 (4.8)	10 (5.2)	0.8 (0.3-2.3)
P_{trend} = 0.55			
T-carrier	80 (37.5)	83 (43.0)	0.8 (0.5-1.2)
LTA IVS1 −82			
G/G	85 (38.8)	76 (37.1)	1.0
C/G	107 (48.9)	102 (49.8)	1.0 (0.6-1.5)
C/C	27 (12.3)	27 (13.1)	0.9 (0.5-1.6)
P_{trend} = 0.91			
C-carrier	134 (61.2)	129 (62.9)	1.0 (0.6-1.4)
LTA IVS1+90			
A/A	90 (39.8)	88 (42.1)	1.0
A/G	101 (44.7)	92 (44.0)	1.2 (0.8-1.8)
G/G	35 (15.5)	29 (13.9)	1.2 (0.7-2.2)
P_{trend} = 0.71			
G-carrier	136 (60.2)	114 (57.9)	1.2 (0.8-1.7)
TNF −308			
G/G	146 (67.3)	139 (68.8)	1.0
A/G	59 (27.2)	57 (28.2)	1.0 (0.7-1.6)
A/A	12 (5.5)	6 (3.0)	1.9 (0.7-5.4)
P_{trend} = 0.46			
A-carrier	71 (32.7)	63 (31.2)	1.1 (0.7-1.7)
PPARG −Pro[12]Ala			
G/G	153 (77.3)	146 (79.4)	1.0
G/C	41 (20.7)	37 (20.1)	1.1 (0.7-1.8)
C/C	4 (2.0)	1 (0.5)	3.2 (0.3-31.1)
P_{trend} = 0.56			
C-carrier	45 (22.7)	38 (21.5)	1.2 (0.7-1.9)
PTGS2 −765			
G/G	151 (71.9)	141 (71.9)	1.0
G/C	54 (25.7)	52 (26.5)	0.9 (0.6-1.4)
C/C	5 (2.4)	3 (1.5)	1.3 (0.3-5.9)
P_{trend} = 0.85			
C-carrier	59 (28.1)	55 (28.0)	0.9 (0.6-1.5)
PTGS2 Ex3 −8			
G/G	154 (73.3)	142 (72.1)	1.0
G/C	52 (24.8)	51 (25.9)	1.0 (0.6-1.6)
C/C	4 (1.9)	4 (2.0)	1.2 (0.3-5.1)
P_{trend} = 0.97			
C-carrier	56 (26.7)	55 (27.9)	1.0 (0.6-1.6)
PTGS2 IVS5 −275			
T/T	149 (71.0)	136 (69.0)	1.0
T/G	54 (25.7)	57 (28.9)	0.8 (0.5-1.3)
G/G	7 (3.3)	4 (2.1)	1.4 (0.4-5.1)
P_{trend} = 0.53			
G-carrier	61 (29.0)	61 (31.0)	0.9 (0.6-1.3)
PTGS2 Ex10 +837			
C/C	92 (43.8)	77 (39.1)	1.0
T/C	88 (41.9)	102 (51.8)	0.7 (0.5-1.1)
T/T	30 (14.3)	18 (9.1)	1.2 (0.6-2.4)
P_{trend} = 0.16			
T-carrier	118 (56.2)	120 (60.9)	0.8 (0.5-1.2)

*ORs adjusted for age, sex, and ethnicity.

colonoscopy, whereas the control group had only undergone sigmoidoscopy. Consequently, it is plausible that some of the controls had undetected polyps in the proximal colon. If the proximal and distal colon share similar etiologies with respect to the genetic factors investigated here, then the data could be biased, with possible attenuation of the findings. However, restriction of analyses to distal adenomas did not yield any material change in the results, suggesting such bias was not relevant here.

IL1β is a prominent proinflammatory cytokine, which together with IL6 and tumor necrosis factor (TNF) serve as primary initiators of the complex inflammatory response. We observed an increased risk of colorectal adenoma among carriers of the *IL1B* −31-C allele. This allele is in linkage disequilibrium with the *IL1B* −1116-T allele, which has been associated with elevated levels of IL1β (2); thus, this allele may be a marker for a proinflammatory phenotype. It is also plausible that both could be in linkage disequilibrium with another, untested variant that influences the levels of IL1β. A previous study investigated the *IL1B* −31-C>T polymorphism in relation to colorectal cancer and did not detect an association (5); however, the *IL1B* −31-C allele has been associated with *H. pylori*−induced gastric cancer (3).

The fact that the genotype distributions for the *IL1B* −31-C>T polymorphism did not conform to Hardy-Weinberg equilibrium was a concern. The quality control data showed complete concordance for this assay, thus making genotyping an unlikely source of error. Comparison of expected genotype frequencies under Hardy-Weinberg equilibrium and observed frequencies among the controls revealed fewer heterozygotes than expected. This raises the possibility that the positive association observed between the *IL1B* −31-C>T genotype and adenoma was due to chance. In an attempt to circumvent this problem, we compared the observed genotype distributions among the cases with the expected distributions among the controls. This yielded attenuated risk estimates for the heterozygous genotype (OR, 0.7; 95% CI, 0.5-1.1) but an enhanced, yet nonsignificant, association for homozygosity for the less common allele (OR, 1.3; 95% CI, 0.7-2.4).

IL8 is a potent chemokine for neutrophils, recruiting them to sites of infection and regulating leukocyte trafficking through peripheral lymphoid tissues. High concentrations of IL8 have been detected in the colonic lumen of ulcerative colitis patients, and rectal dialysate from these patients is capable of activating neutrophils *in vitro* (23). Three well-characterized SNPs in the 5′-promoter sequence have been analyzed and shown to alter expression of the gene under laboratory conditions (24). Specifically, there is evidence to suggest enhanced IL8 expression among carriers of the *IL8* −251-A allele (22). In our study, we observed a 3-fold increase in risk for colorectal adenoma among *IL8* −251-A/A carriers. Interestingly, a previous colorectal cancer study conducted among a Spanish population found an inverse association between carriage of the *IL8* −251-A allele and colorectal cancer (4); however, studies on other cancers of the gastrointestinal tract have found reported positive associations between the *IL8* −251-A allele and disease risk (25).

We detected an increased risk of adenoma for carriers of the *IL10* −819-T/T genotype among nonusers of NSAIDs, whereas this association was not apparent among those reporting regular use. Because IL10 has been implicated in colorectal carcinogenesis in murine models (26), and because the *IL10* −819-T allele has been linked to lower levels of IL10 (27, 28), it is plausible that this allele is associated with increased levels of basal inflammation in the colon, which is modified by NSAID use. This is consistent with the findings of the previous study of *IL10* polymorphisms and colorectal cancer, in which carriers of the *IL10* −626-A allele, which has been associated with lower levels of IL10, were at reduced risk of colorectal cancer but only among habitual users of aspirin (5). It is also possible that due to the potent anti-inflammatory effect of NSAIDs, the association of cytokine polymorphisms with colorectal adenoma is masked among NSAID users, and the current data are consistent with this. It should also be noted that due to the small size of the study, we have limited statistical power to detect interactions.

There was suggestion that the *IL6* −174 G/G, *IL10* −819 T/T, *TNF* −308 A/A, and *LTA* IVS1 +90 G/G genotypes were associated with colorectal adenoma, although the ORs did not quite attain statistical significance. Based on functional data, these genotypes predict elevated expression of these proinflammatory cytokines; thus, these data are also supportive of the notion that a proinflammatory genetic profile is a positive risk factor for colorectal neoplasia. Interestingly, although the *PPARG* ala/ala genotype was rare in this population, four adenoma cases were carriers compared with one control. The lack of association observed for the remaining polymorphisms may not only reflect the small sample size but may also indicate that the genes (or SNPs) studied are not relevant for colorectal tumors.

In conclusion, we have observed that SNPs in key cytokine genes could be important risk factors for colorectal adenoma. Specifically, we observed several SNPs in *IL1B*, *IL8*, and *IL10*, known to alter risk or functional expression of the gene that seemed to confer increased susceptibility to colorectal adenomas. Further analysis of the untested SNPs in these genes, either by haplotype analysis or a pairwise comparison approach, is needed to confirm the current findings. Moreover, a more thorough analysis of common variants in these genes and related genes in the same pathways of inflammation are required to determine the contribution of inflammatory genes to colorectal adenomas, precursor lesions to colorectal carcinoma. Our data support the contention that large, well-planned studies that examine inflammation-related genes in relation to the colorectal adenoma-carcinoma sequence could uncover important mechanistic pathways and could lead to new intervention or prevention strategies.

References

1. Macdonald TT, Monteleone G. Immunity, inflammation, and allergy in the gut. Science 2003;307:1920–5.
2. Hwang IR, Kodama T, Kikuchi S, et al. Effect of interleukin 1 polymorphisms on gastric mucosal interleukin 1beta production in *Helicobacter pylori* infection. Gastroenterology 2002;123:1793–803.
3. El-Omar EM, Carrington M, Chow WH, et al. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. Nature 2000;404:398–402.
4. Landi S, Moreno V, Gioia-Patricola L, et al. Association of common polymorphisms in inflammatory genes interleukin (IL)6, IL8, tumor necrosis factor alpha, NFKB1, and peroxisome proliferator-activated receptor gamma with colorectal cancer. Cancer Res 2003;63:3560–6.
5. Macarthur M, Sharp L, Hold GL, Little J, El-Omar EM. The role of cytokine gene polymorphisms in colorectal cancer and their interaction with aspirin use in the northeast of Scotland. Cancer Epidemiol Biomarkers Prev 2005;14:1613–8.
6. Jones R, Adel-Alvarez LA, Alvarez OR, Broaddus R, Das S. Arachidonic acid and colorectal carcinogenesis. Mol Cell Biochem 2003;253:141–9.
7. Romano M, Claria J. Cyclooxygenase-2 and 5-lipoxygenase converging functions on cell proliferation and tumor angiogenesis: implications for cancer therapy. FASEB J 2003;17:1986–95.
8. Ulrich CM, Whitton J, Yu JH, et al. PTGS2 (COX-2) −765G > C promoter variant reduces risk of colorectal adenoma among nonusers of nonsteroidal anti-inflammatory drugs. Cancer Epidemiol Biomarkers Prev 2005;14:616–9.
9. Lin HJ, Lakkides KM, Keku TO, et al. Prostaglandin H synthase 2 variant (Val^{511}Ala) in African Americans may reduce the risk for colorectal neoplasia. Cancer Epidemiol Biomarkers Prev 2002;11:1305–15.
10. Ali IU, Luke BT, Dean M, Greenwald P. Allellic variants in regulatory regions of cyclooxygenase-2: association with advanced colorectal adenoma. Br J Cancer 2005;93:953–9.
11. Koh WP, Yuan JM, van den Berg D, Lee HP, Yu MC. Interaction between cyclooxygenase-2 gene polymorphism and dietary n-6 polyunsaturated fatty acids on colon cancer risk: the Singapore Chinese Health Study. Br J Cancer 2004;90:1760–4.
12. Cox DG, Pontes C, Guino E, et al. Polymorphisms in prostaglandin synthase 2/cyclooxygenase 2 (PTGS2/COX2) and risk of colorectal cancer. Br J Cancer 2004;91:339–43.
13. Welch JS, Ricote M, Akiyama TE, Gonzalez FJ, Glass CK. PPARgamma and PPARdelta negatively regulate specific subsets of lipopolysaccharide and IFN-gamma target genes in macrophages. Proc Natl Acad Sci U S A 2003;100:6712–7.
14. Wada K, Nakajima A, Blumberg RS. PPARgamma and inflammatory bowel disease: a new therapeutic target for ulcerative colitis and Crohn's disease. Trends Mol Med 2001;7:329–31.
15. Chinetti G, Fruchart JC, Staels B. Peroxisome proliferator-activated receptors (PPARs): nuclear receptors at the crossroads between lipid metabolism and inflammation. Inflamm Res 2000;49:497–505.
16. Tanaka T, Kohno H, Yoshitani S, et al. Ligands for peroxisome proliferator-activated receptors alpha and gamma inhibit chemically induced colitis and formation of aberrant crypt foci in rats. Cancer Res 2001;61:2424–8.
17. Gong Z, Xie D, Deng Z, et al. The PPAR{gamma} Pro^{12}Ala polymorphism and risk for incident sporadic colorectal adenomas. Carcinogenesis 2005;26:579–85.
18. Sinha R, Chow WH, Kulldorff M, et al. Well-done, grilled red meat increases the risk of colorectal adenomas. Cancer Res 1999;59:4320–4.
19. Sinha R, Kulldorff M, Gunter MJ, Strickland P, Rothman N. Dietary benzo[a]pyrene intake and risk of colorectal adenoma. Cancer Epidemiol Biomarkers Prev 2005;14:2030–4.
20. Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. Am J Hum Genet 2001;68:978–89.
21. Wacholder S, Chanock S, Garcia-Closas M, El Ghormli L, Rothman N. Assessing the probability that a positive report is false: an approach for molecular epidemiology studies. J Natl Cancer Inst 2004;96:434–42.
22. Hull J, Ackerman H, Isles K, et al. Unusual haplotypic structure of IL8, a susceptibility locus for a common respiratory virus. Am J Hum Genet 2001;69:413–9.
23. Keshavarzian A, Fusunyan RD, Jacyno M, Winship D, MacDermott RP, Sanderson IR. Increased interleukin-8 (IL-8) in rectal dialysate from patients with ulcerative colitis: evidence for a biological role for IL-8 in inflammation of the colon. Am J Gastroenterol 1999;94:704–12.
24. Hull J, Thomson A, Kwiatkowski D. Association of respiratory syncytial virus bronchiolitis with the interleukin 8 gene region in UK families. Thorax 2000;55:1023–7.

25. Savage SA, Abnet CC, Mark SD, et al. Variants of the IL8 and IL8RB genes and risk for gastric cardia adenocarcinoma and esophageal squamous cell carcinoma. Cancer Epidemiol Biomarkers Prev 2004;13:2251–7.
26. Kuhn R, Lohler J, Rennick D, Rajewsky K, Muller W. Interleukin-10-deficient mice develop chronic enterocolitis. Cell 1993;75:263–74.
27. Crawley E, Kay R, Sillibourne J, Patel P, Hutchinson I, Woo P. Polymorphic haplotypes of the interleukin-10 5′ flanking region determine variable interleukin-10 transcription and are associated with particular phenotypes of juvenile rheumatoid arthritis. Arthritis Rheum 1999;42:1101–8.
28. Temple SE, Lim E, Cheong KY, et al. Alleles carried at positions -819 and -592 of the IL10 promoter affect transcription following stimulation of peripheral blood cells with *Streptococcus pneumoniae*. Immunogenetics 2003; 55:629–32.
29. Rosenwasser LJ, Klemm DJ, Dresback JK, et al. Promoter polymorphisms in the chromosome 5 gene cluster in asthma and atopy. Clin Exp Allergy 1995; 25 Suppl 2:74–8; discussion 95–6.
30. Song Z, Casolaro V, Chen R, Georas SN, Monos D, Ono SJ. Polymorphic nucleotides within the human IL-4 promoter that mediate overexpression of the gene. J Immunol 1996;156:424–9.
31. Fishman D, Faulds G, Jeffery R, et al. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. J Clin Invest 1998;102:1369–76.
32. van der Pouw Kraan TC, Kucukaycan M, Bakker AM, et al. Chronic obstructive pulmonary disease is associated with the −1055 IL-13 promoter polymorphism. Genes Immun 2002;3:436–9.
33. Wilson AG, de Vries N, Pociot F, di Giovine FS, van der Putte LB, Duff GW. An allelic polymorphism within the human tumor necrosis factor alpha promoter region is strongly associated with HLA A1, B8, and DR3 alleles. J Exp Med 1993;177:557–60.
34. Wu WS, McClain KL. DNA polymorphisms and mutations of the tumor necrosis factor-alpha (TNF-alpha) promoter in Langerhans cell histiocytosis (LCH). J Interferon Cytokine Res 1997;17:631–5.
35. Deeb SS, Fajas L, Nemoto M, et al. A Pro12Ala substitution in PPARgamma2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. Nat Genet 1998;20:284–7.
36. Papafili A, Hill MR, Brull DJ, et al. Common promoter variant in cyclooxygenase-2 represses gene expression: evidence of role in acute-phase inflammatory response. Arterioscler Thromb Vasc Biol 2002;22: 1631–6.